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Comprehensive genomic profiling of lung cancer using a validated panel to explore therapeutic targets in East Asian patients

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Key words

Actionable genomic alteration, comprehensive genomic profiling, lung cancer, next generation sequencing, targeted therapy

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People of East Asian ethnicity have a different prevalence of and show unique clinical characteristics and tumor histology of oncogenic mutations. However, only limited studies have explored the landscape of genomic alterations in lung adenocarcinoma derived from Asian patients thus far. In this single-center study, with an aim to elucidate the mutational profile of lung cancer in people of Chinese ethnicity and to use the obtained information to guide decision-making for treatment, we employed a well-validated assay to perform comprehensive genomic characterization of tumor specimens from 306 Chinese lung cancer patients. A total of 845 individual genomic alterations were found in 145 tumor-related genes with a median of 2.8 alterations (range: 1–18) per sample. The most frequently mutated genes were *EGFR* (46.7%), *TP53* (21.2%), *ALK* (12.1%; 8.8% of mutation and 3.3% of rearrangement) and *KRAS* (10.1%). Upon comparison with the Cancer Genome Atlas dataset, we found that *EGFR* was mutated at a much higher frequency in our cohort than in Caucasians, whereas *KRAS* was only found in 10.1% of our Chinese patients. Clinically relevant genomic alterations were identified in 185 (60.5%) patients, including 50% in adenocarcinoma patients and 14% in squamous cell carcinoma patients. Our findings suggest that the Asian ethnicity is significantly different from the Caucasian ethnicity with regard to the presence of somatic driver mutations. Furthermore, we showed that the use of a comprehensive genotyping approach could help identify actionable genomic alterations that have potential impact on therapeutic decisions.

The development of targeted therapy has dramatically changed the treatment modalities for non-small-cell lung cancer (NSCLC) in specific genotypic subsets of patients.⁽¹⁾ The most recent version of the National Comprehensive Cancer Network (NCCN) guidelines for NSCLC recommends that in addition to the routine testing conducted for *EGFR*, *KRAS* and *ALK*, tests should also be conducted for *BRAF* and *ERBB2*

mutations, *MET* amplifications and exon 14 skipping mutations, and gene rearrangements involving *ROS1* and *RET*.⁽²⁾ Given the increasing availability of various targeted therapies, combined with the maturity of new technologies, comprehensive genomic profiling (CGP) of lung cancer is rapidly becoming an important trend in cancer pathology diagnosis.^(3–6) Comprehensive information regarding tumor-specific molecular

abnormalities is valuable in choosing suitable treatment options to maximize therapeutic benefits and minimize therapy-associated risks.^(7–9) Moreover, comprehensive analysis of mutations in oncogenes and key cancer pathways is necessary to understand the molecular basis of drug resistance and to modify treatment options accordingly.⁽¹⁰⁾ Finally, detailed profiling of these aberrations in tumors will improve our understanding of the genetic basis of diseases and aid in prognostication.^(11–13)

Previous studies have confirmed the feasibility of routine multiplex genotyping in patients with lung adenocarcinomas (ADC) for selecting matched therapies and trials.^(14–16) Many patients could become eligible for targeted therapy due to the discovery of clinically actionable genomic alterations via next generation sequencing (NGS)-based assays.^(17,18) More importantly, it has been found that individuals with an actionable driver receiving matched target agents show an obvious improvement in median survival over those who do not receive targeted therapy.^(19–21) However, most of these previous studies have focused on tumor samples from Caucasian populations. It is well known that people of Asian ethnicity have a different prevalence of and show unique clinical characteristics and tumor histology of oncogenic mutations.⁽²²⁾ One example is that female never-smokers of Asian ethnicity show a higher frequency of *EGFR* mutation than Caucasian female never-smokers.^(23,24) Therefore, there is a clear need for a more comprehensive profiling of oncogenic mutations in the Asian population to guide diagnosis and therapies for lung cancer in patients of this ethnicity. In this study, we used a well-validated assay to perform comprehensive genomic profiling on tumor specimens from 306 Chinese lung cancer patients with the aim to elucidate the mutational profile of NSCLC in people of Chinese ethnicity and to use the obtained information to guide decision-making during treatment.

Materials and Methods

Patients and samples. Formalin-fixed paraffin-embedded (FFPE) specimens were obtained from 306 Chinese patients with lung cancer who underwent either surgical resection or biopsy from June 2016 to December 2016 at the First Affiliated Hospital of Guangzhou Medical University. The specimens were independently reviewed by two pathologists to confirm the histological subtype and tumor cell content. Other relevant clinical and pathological information, including smoking history, were also collected. The present study was approved by the Institutional Review Board of the First Affiliated Hospital of Guangzhou Medical University. All the patients who participated in this study provided written informed consent. All the molecular tests were conducted in accredited clinical genetics laboratories.

Histological analysis. The pathologic records of the specimens and all available HE-stained tissue sections, in addition to any available sections with special stains or immunohistochemical analysis, were reviewed. Pathological information was collected, including maximum tumor sizes (in cm) and pathologic disease stages (p-stage). Staging was based on the guidelines of the 7th edition of the TNM classification for lung cancer. All the available HE-stained sections, for each case, were examined by two pathologists. Histological classification was according to the IASLC/ATS/ERS classification of lung ADC; each histologic component present was recorded in 5% increments. The tumors were classified as ADC in situ (AIS), minimally invasive ADC (MIA), and invasive ADC, which were further classified into

lepidic predominant, papillary predominant, acinar predominant, solid predominant, micropapillary predominant, invasive mucinous ADC (IMA), and others, according to the predominant histologic component. The amount of lepidic growth and assessment of the presence or absence of stromal, lymphovascular space and pleural invasion are the important factors in the diagnosis of AIS, MIA and invasive ADC.

Next generation sequencing-based genomic profiling. The specimens were reviewed to ensure tissue adequacy (>20% tumor nuclei) before testing. DNA was extracted from unstrained FFPE resections using the QIAamp DNA FFPE Tissue Kit following the manufacturer's instructions (Qiagen, Hilden, Germany). DNA concentration was measured using a Qubit fluorometer (Thermo Fisher, Waltham, MA, USA). A targeted next-generation sequencing method was used to identify the clinically relevant mutation profiles as described previously.⁽²⁵⁾ Briefly, FFPE DNA was used for library construction. Hybridization capture of 13 introns and 436 exons from 145 cancer-related genes (Table S1), including recurrent rearrangement and amplification, was performed. The hybrid capture libraries were then sequenced to >500× average unique coverage using Ion Proton Sequencers (Thermo Fisher). Sequencing data were processed using a customized bioinformatics pipeline named Otype, which was designed to simultaneously detect single nucleotide variations (SNV), short insertions and deletions (InDels), copy number variations (CNV) and gene rearrangements. Finally, data interpretation was focused on genomic alterations associated with clinically available targeted treatment options according to the standards and guidelines of the NCCN, the Association for Molecular Pathology (AMP), the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP).⁽²⁶⁾

Statistical analysis. Statistical analysis was performed using R studio 19.0 (RStudio, Boston, MA, USA) and IBM SPSS Statistics 22.0 (SPSS, Chicago, IL, USA). The χ^2 -test, the *t*-test and Fisher's exact test were used to analyze the associations of mutational status with clinical characteristics. The association between driver gene alterations and ADC subtypes was analyzed using a logistic model adjusted for age, gender and smoking status. A two-tailed *P*-value of <0.05 was considered statistically significant.

Results

Patient characteristics. A total of 320 patients who underwent either biopsy or surgery for lung cancer between June 2016 and December 2016 were enrolled for this study. Fourteen patients were excluded because of incomplete clinicopathological data (*n* = 6), non-lung primary tumor (*n* = 3) and insufficient tumor tissue (*n* = 5). The demographic and histopathological features of the remaining 306 patients included in the study are shown in Table 1. Regarding the histologic subtype, 255 cases (83.3%) were lung ADC, 34 (11.1%) were SCC and 17 (5.6%) were lung cancer not otherwise specified (NOS). The median age of the patients was 59 years (range: 21–82 years). A total of 144 (47.1%) patients were female and 195 (63.7%) were never-smokers. Three (1%) specimens were derived from tissues obtained at tumor biopsy, and others were from surgically resected tissue. According to the 7th edition of American Joint Committee on Cancer TNM staging, 215 patients (70.3%) were classified as stage I and II, and 91 patients (29.7%) as stage III and IV.

Genomic alterations. Both biopsy and surgical specimens yielded sufficient DNA for hybrid capture-based NGS assay.

Table 1. Clinicopathological characteristics of studied patients

Characteristic	Number	(%)
Gender		
Male	162	52.9
Female	144	47.1
Age		
Median	59	
Range	21–82	
Smoker		
Never	195	63.7
Ever	111	36.3
Clinical stage		
I & II	215	70.3
III & IV	91	29.7
Histology type		
Adenocarcinoma	255	83.3
Squamous cell carcinoma	34	11.1
NOS	17	5.6

NOS, not otherwise specified.

The average depth of the target exceeded 696-fold, and more than 98.86% of bases had at least 20-fold coverage (Fig. S1). In the 306 samples tested, 845 individual genomic alterations were found in 145 tumor-related genes, with a median of 2.8 alterations (range: 1–18) per sample. One or more genomic alterations were identified in tumors from 92.8% (284 of 306) of the patients, including 240 of the 255 (94.1%) individuals with ADC, 32 of the 34 (94.1%) patients with SCC, and 12 of the 17 (70.6%) patients with NOS. The distribution of driver mutations is shown in Figure 1. The most frequently mutated

genes were *EGFR* (143 of 306, 46.7%), *TP53* (65 of 306, 21.2%), *ALK* (37 of 306, 12.1%) and *KRAS* (31 of 306, 10.1%), which have all been reported as well-known driver genes of lung cancer. The other frequently mutated genes included *EZH2* (8.8%), *NOTCH1* (7.8%) and *RBM10* (7.2%), *ESR1* (5.2%), *RET* (4.9%) and *ERBB2* (4.5%). The majority of SNV and InDels were in *EGFR*, *TP53* and *KRAS*. Gene rearrangements most commonly involved *ALK* (3.3%, 10 of 306) and *ROS1* (1.3%, 4 of 306). Among the 10 *ALK* gene rearrangements and three *MET* amplifications detected using the NGS assay, 12 (92.3%) showed consistent results with the conventional test either by IHC or FISH, whereas one showed an inconsistent result (Table S2).

Correlations between driver mutations and clinicopathological characteristics. Correlations of genotype with clinicopathological characteristics are listed in Table 2. The *EGFR* mutation rate was significantly higher in women than in men (61.8% vs 33.3%, $P < 0.001$) and in patients with ADC than in those with SCC and NOS (54.1% vs 8.8% and 11.8, $P < 0.001$). No association was found between *EGFR* mutation status and the patients' age, smoking history and tumor stage. In contrast, the *KRAS* mutation rate was significantly higher in men than in women (16.0% vs 3.5%, $P < 0.001$) and in ever-smokers than in never-smokers (17.1% vs 6.2%, $P = 0.004$). No association was found between *ALK* rearrangement and clinicopathological characteristics.

Driver mutation status in histopathologic subtypes of adenocarcinomas. Next, we aimed to investigate associations between mutation status (*EGFR* and *KRAS*) and the new classification in our Asians cohort. We excluded patients for whom predominant histology subtype could not be determined (6 of 255, 2.4%) and those with metastatic lung adenocarcinomas

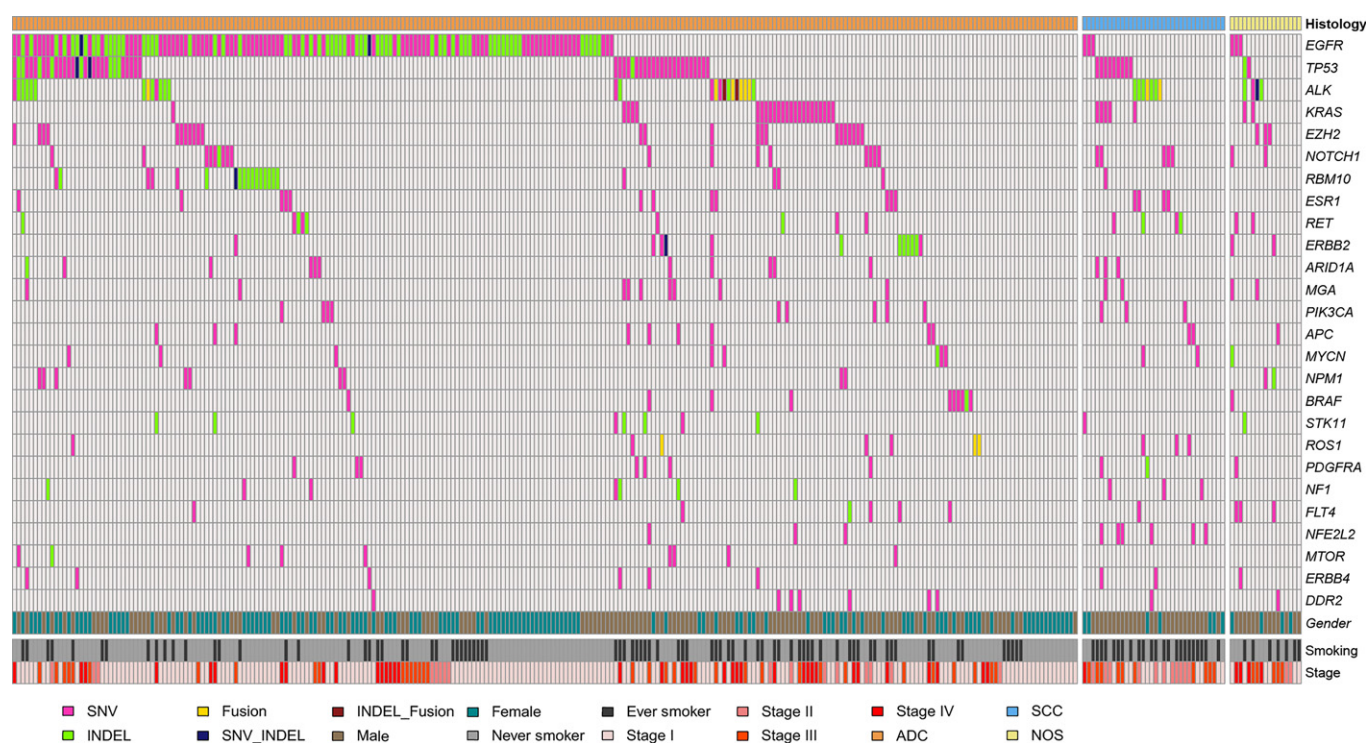


Fig. 1. Significantly mutated genes and clinicopathological features of 306 patients with lung cancer tumors. Figure shows genes mutated in at least 3% of the patients. Each column represents the cancer profile in one patient. Samples were sorted by tumor histology subtype, gender, smoking history, and tumor stage distinguished by color. ADC, adenocarcinoma; INDEL, short insertions and deletions; NOS, not otherwise specified; SCC, squamous cell carcinoma; SNV, single nucleotide variations.

Table 2. Correlation of *EGFR* and *KRAS* mutations and *ALK* rearrangements with clinicopathological features

Features	<i>EGFR</i> mutation			<i>KRAS</i> mutation			<i>ALK</i> gene rearrangement		
	Wild type (%)	Mutant (%)	<i>P</i> † value	Wild type (%)	Mutant (%)	<i>P</i> † value	Wild type (%)	Mutant (%)	<i>P</i> † value
Gender									
Male	108 (66.7)	54 (33.3)	<0.001	136 (84.0)	26 (16.0)	<0.001	158 (97.5)	4 (2.5)	0.525
Female	55 (38.2)	89 (61.8)		139 (96.5)	5 (3.5)		138 (95.8)	6 (4.1)	
Age									
Mean (SD)	57.7 (11.8)	57.6 (10.4)	0.963	57.4 (11.5)	60.3 (6.8)	0.045	57.8 (11.1)	54.1 (11.2)	0.332
Smoking history									
Never	83 (42.6)	112 (57.4)	0.318	183 (93.8)	12 (6.2)	0.004	189 (96.9)	6 (3.1)	1.000
Ever	40 (36.0)	71 (64.0)		92 (82.9)	19 (17.1)		107 (96.4)	4 (3.6)	
Stage									
I & II	109 (50.7)	106 (49.3)	0.207	197 (91.6)	18 (8.4)	0.174	211 (98.1)	4 (1.9)	0.070
III & IV	54 (59.3)	37 (40.7)		78 (85.7)	13 (14.3)		85 (93.4)	6 (6.6)	
Histology type									
ADC	117 (45.9)	138 (54.1)	<0.001	231 (90.6)	24 (9.4)	0.359	247 (96.9)	8 (3.1)	0.333
SCC	31 (91.2)	3 (8.8)		29 (85.3)	5 (14.7)		32 (94.1)	2 (5.9)	

† χ^2 -test or Fisher's exact test as appropriate. ADC, adenocarcinoma; SCC, squamous cell carcinoma.

(14 of 255, 5.5%). The histopathologic assessment according to the IASLC/ATS/ERS classification showed that 9 (3.8%) were AIS, 32 (13.6%) were MIA, 111 (47.2%) were acinar predominant, 17 (7.2%) were lepidic predominant, 25 (10.6%) were papillary predominant, 10 (4.3%) were micropapillary predominant, 26 (11.1%) were solid predominant and 5 (2.1%) were IMA.

EGFR mutations were positively correlated with acinar predominant tumors ($P = 0.001$) and negatively correlated with solid predominant tumors ($P = 0.023$) (Table 3). Among the 235 cases, the frequency of *EGFR* mutation in the cases of AIS, MIA, acinar predominant, lepidic predominant, papillary predominant, micropapillary predominant, solid predominant and IMA was 33.3%, 50.0%, 67.6%, 64.7%, 64.0%, 20.0%, 26.9% and 0%, respectively (Fig. 2).

KRAS mutations were most prevalent in IMA (60.0%), followed by micropapillary predominant (20.0%), solid predominant (15.4%), lepidic predominant (11.8%), AIS (11.1%), acinar predominant (7.2%), papillary predominant (4.0%) and MIA (0%) (Fig. 2). The frequency of *KRAS* mutations was positively correlated with IMA ($P = 0.013$) (Table 3).

Comparison between East Asians and Caucasians. To compare the frequency of driver mutations of ADC between East Asians

and Caucasians, we obtained all the available ADC cases (501) from The Cancer Genome Atlas (TCGA) dataset. Notable differences from TCGA data included *EGFR* (54.5% vs 15.0%, $P < 0.001$), *KRAS* (9.8% vs 33.7%; $P < 0.001$), *TP53* (21.2% vs 54.1%, $P < 0.001$), *ALK* (10.2% vs 5.8%, $P = 0.027$), *EZH2* (9.4% vs 2.2%, $P < 0.001$), *ERBB2* (5.5% vs 2.4%, $P = 0.027$), *MGA* (3.5% vs 7.6%, $P = 0.029$), *MYCN* (3.1% vs 1.0%, $P = 0.032$), *NPM1* (3.5% vs 1.0%, $P = 0.015$), *BRAF* (3.9% vs 8.4%, $P = 0.022$), *SKT11* (3.1% vs 16.6%, $P < 0.001$), *PDGFRA* (2.7% vs 7.0%, $P = 0.016$), *NF1* (2.7% vs 11.6%, $P < 0.001$) and *ERBB4* (2.4% vs 8.4%, $P = 0.001$). The full comparison of selected gene alteration frequencies between two cohorts is depicted in Figure 3a and Table 4.

For *EGFR* mutation, missense mutation in exon 21 was more frequently observed in East Asians (57.6% vs 37.3%, $P = 0.005$), and exon 18 missense mutation (1.4% vs 8.0%, $P = 0.016$) and mutations in exon 20 (1.4% vs 10.7%, $P = 0.002$) were more frequently observed in Caucasians. No statistically significant differences are observed in deletion and insertions in exon 19 (39.6 vs 41.3%) and T790M on exon 20 (0.7% vs 2.7%). For *KRAS* mutation, comparing to Caucasians, statistically significant differences were found in G12D in

Table 3. Correlation of *EGFR* and *KRAS* with histopathologic subtypes of new adenocarcinoma classification

Features	<i>EGFR</i> mutation			<i>KRAS</i> mutation		
	Wild type (%)	Mutant (%)	<i>P</i> †	Wild type (%)	Mutant (%)	<i>P</i> †
AIS	6 (66.7)	3 (33.3)	0.148	8 (88.9)	1 (11.1)	0.255
MIA	16 (50.0)	16 (50.0)	0.250	32 (100.0)	0 (0)	0.998
Acinar	36 (32.4)	75 (67.6)	0.001	103 (92.8)	8 (7.2)	0.446
Lepidic	6 (35.3)	11 (64.7)	0.517	15 (88.2)	2 (11.8)	0.637
Papillary	9 (36.0)	16 (64.0)	0.313	24 (96.0)	1 (4.0)	0.347
MP	8 (80.0)	2 (20.0)	0.210	8 (80.0)	2 (20.0)	0.554
Solid	19 (73.1)	7 (26.9)	0.023	22 (84.6)	4 (15.4)	0.723
IMA	5 (100.0)	0 (0.0)	0.999	2 (40.0)	3 (60.0)	0.013

†Logistic model adjusted for age, gender and smoking status. AIS, adenocarcinoma in situ; IMA, invasive mucinous adenocarcinoma; MIA, minimally invasive adenocarcinoma; MP, micropapillary adenocarcinoma.

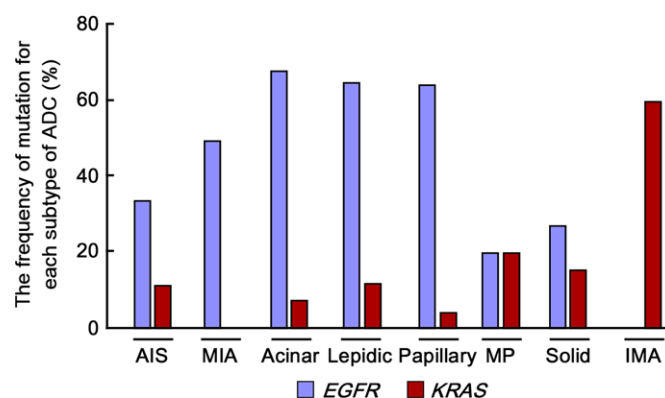


Fig. 2. Gene mutation detection rates for each histological subtype of adenocarcinoma (ADC). AIS, adenocarcinoma in situ; IMA, invasive mucinous adenocarcinoma; MIA, minimally invasive adenocarcinoma; MP, Micropapillary adenocarcinoma.

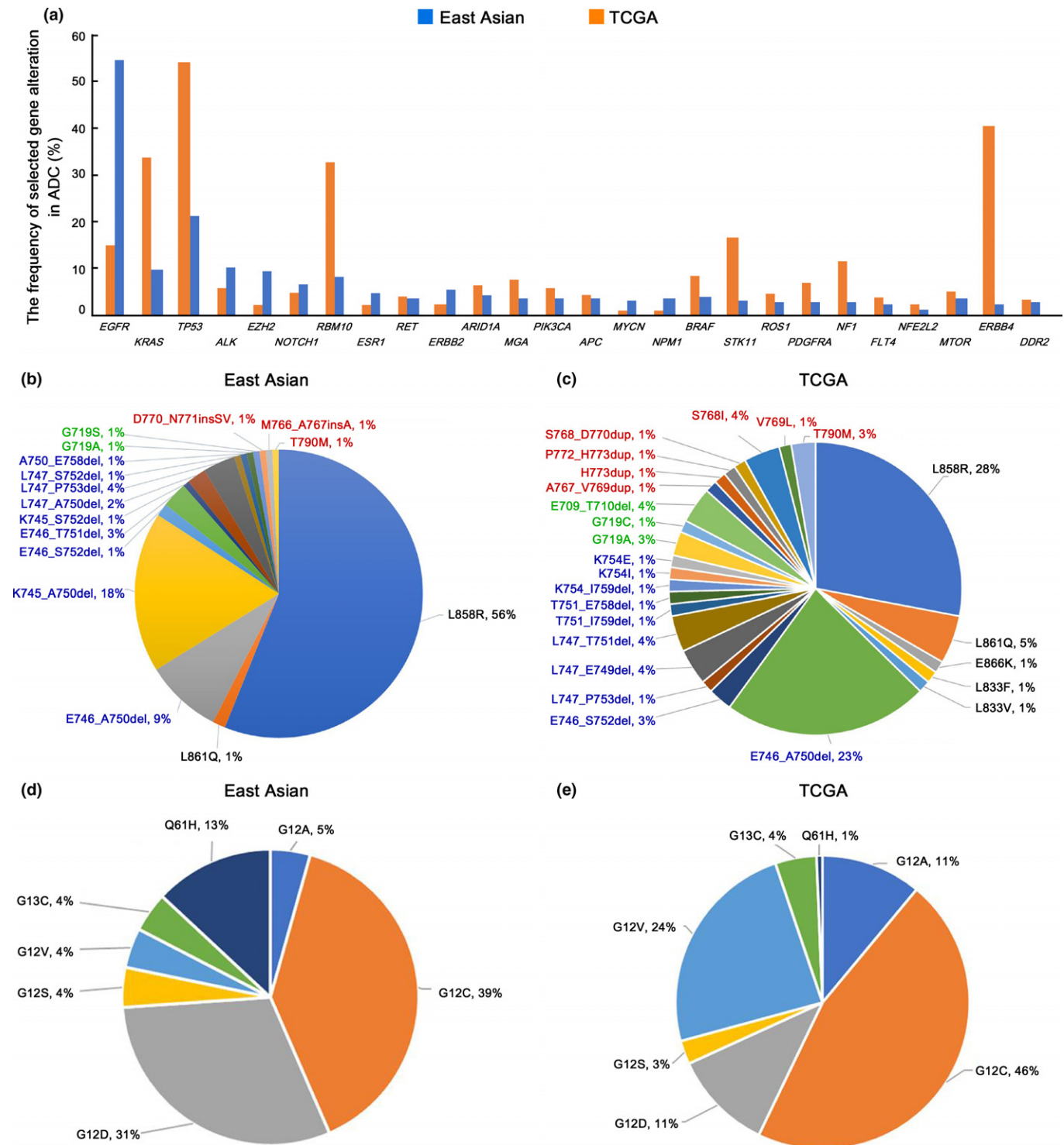


Fig. 3. Comparison of selected gene mutations in adenocarcinoma (ADC) between East Asians with Caucasians. (a) Comparison of selected gene alteration frequencies in East Asian and TCGA cohorts. Selected gene mutated in at least 3% of the East Asians cohort. (b) Gene mutation detection rates for *EGFR* in East Asian cohort. (c) Gene mutation detection rates for *EGFR* in TCGA. *EGFR* exon 18 mutations shown in green font; *EGFR* exon 19 mutations shown in blue font; *EGFR* exon 20 mutations shown in red font; *EGFR* exon 21 mutations shown in black font. (d) Gene mutation detection rates for *KRAS* in East Asian cohort. (e) Gene mutation detection rates for *KRAS* in TCGA. TCGA, The Cancer Genome Atlas.

Asians (28.0% vs 10.1%, $P = 0.011$) and in Q61H (16.0% vs 0.6%, $P < 0.001$).

Clinically relevant genomic alterations. Based on the recent guidelines of NCCN, AMP, ASCO, and CAP, clinically relevant genomic alterations were identified in 191 (62%) patients

(Table 5). Among the 255 patients with ADC, 174 (68%) harbored an actionable alteration, whereas only 13 (38%) of the 34 patients with SCC did so. As shown in Table 5, the clinically relevant alterations with level I included *EGFR* mutations (140, 45.8%), *KRAS* mutations (31, 10.1%), *ALK* rearrangements (10,

Table 4. Comparison of driver gene mutations of lung adenocarcinoma between East Asian patients and the Caucasian cohort in TCGA dataset

	East Asians (255)		Caucasians (501)		<i>P</i> ^a
	Wild type (%)	Mutant (%)	Wild type (%)	Mutant (%)	
<i>EGFR</i>	139 (54.5)	116 (45.5)	424 (84.6)	77 (15.4)	<0.001
<i>KRAS</i>	25 (9.8)	230 (90.2)	169 (33.7)	332 (66.3)	<0.001
<i>TP53</i>	201 (78.8)	54 (21.2)	230 (45.9)	271 (54.1)	<0.001
<i>ALK</i>	229 (89.8)	26 (10.2)	472 (94.2)	29 (5.8)	0.027
<i>EZH2</i>	231 (90.6)	24 (9.4)	490 (97.8)	11 (2.2)	<0.001
<i>NOTCH1</i>	238 (93.3)	17 (6.7)	477 (95.2)	24 (4.8)	0.282
<i>RBM10</i>	234 (91.8)	21 (8.2)	467 (93.2)	34 (6.8)	0.468
<i>ESR1</i>	243 (95.3)	12 (4.7)	490 (97.8)	11 (2.2)	0.057
<i>RET</i>	246 (96.5)	9 (3.5)	481 (96.0)	20 (4.0)	0.754
<i>ERBB2</i>	241 (94.5)	14 (5.5)	489 (97.6)	12 (2.4)	0.027
<i>ARID1A</i>	244 (95.7)	11 (4.3)	469 (93.6)	32 (6.4)	0.245
<i>MGA</i>	246 (96.5)	9 (3.5)	463 (92.4)	38 (7.6)	0.029
<i>PIK3CA</i>	246 (96.5)	9 (3.5)	472 (94.2)	29 (5.8)	0.179
<i>APC</i>	246 (96.5)	9 (3.5)	479 (95.6)	22 (4.4)	0.572
<i>MYCN</i>	247 (96.9)	8 (3.1)	496 (99.0)	5 (1.0)	0.032
<i>NPM1</i>	246 (96.5)	9 (3.5)	496 (99.0)	5 (1.0)	0.015
<i>BRAF</i>	245 (96.1)	10 (3.9)	459 (91.6)	42 (8.4)	0.022
<i>SKT11</i>	247 (96.9)	8 (3.1)	418 (83.4)	83 (16.6)	<0.001
<i>ROS1</i>	248 (97.3)	7 (2.7)	478 (95.4)	23 (4.6)	0.197
<i>PDGFRA</i>	248 (97.3)	7 (2.7)	466 (93.0)	35 (7.0)	0.016
<i>NF1</i>	248 (97.3)	7 (2.7)	443 (88.4)	58 (11.6)	<0.001
<i>FLT4</i>	249 (97.6)	6 (2.4)	482 (96.2)	19 (3.8)	0.295
<i>NFE2L2</i>	252 (98.8)	3 (1.2)	489 (97.6)	12 (2.4)	0.256
<i>MTOR</i>	246 (96.5)	9 (3.5)	476 (95.0)	25 (5.0)	0.360
<i>ERBB4</i>	249 (97.6)	6 (2.4)	459 (91.6)	42 (8.4)	0.001
<i>DDR2</i>	248 (97.3)	7 (2.7)	484 (96.6)	17 (3.4)	0.631

^aChi-square test was used.

3.3%) and *ROS1* rearrangements (4, 1.3%). *EGFR* was the most frequently mutated gene and mutations in *EGFR* were detected in 46.7% (143 of 306) of the cases. Exon 19 deletions (54 of 143, 37.8%) and exon 21 L858R point mutation (79 of 143, 55.2%) accounted for 93.0% of all the detected *EGFR* mutations. Other *EGFR* mutations included G719X (*n* = 2) on exon 18, M766_A767insASV (*n* = 1), D770_N771insSVD (*n* = 1) and T790M (*n* = 1) on exon 20, L861Q (*n* = 2) on exon 21, and gene amplifications (*n* = 3).

In addition, patients with level II genomic alterations, for which targeted therapy could be considered in phase II/III clinical trials, were found in five patients (1.6%). These included the following alterations: *NRAS* mutation (*n* = 1), *MET* amplification (*n* = 3) and *ERBB2* insertion (*n* = 1) (Table 5).

Finally, there were eight patients with level III and IV genomic alterations, including *BRAF* V600E mutation (*n* = 2), *PIK3CA* H1047X mutation (*n* = 3) and *EGFR* amplification (*n* = 3) (Table 5), which indicate sensitivity or resistance to therapies approved by the FDA or to those included in the professional guidelines for other cancers. We also found drivers in two genes in seven tumors (2.2%). Gene pairings and specific mutations for these patients are presented in Table S3.

Discussion

Lung cancer is the most common cancer and the leading cause of cancer-related deaths in China. Approximately 700 000 new

cases of lung cancer are reported every year in China. Over 300 000 patients with advanced non-squamous NSCLC are expected to be screened for *EGFR* mutations and *ALK* rearrangements according to current guidelines.^(39,40) In the present study, we successfully used a well-validated NGS assay to perform comprehensive genomic profiling on tumor specimens from 306 Chinese lung cancer patients. To our knowledge, this study is the largest in China to demonstrate the successful implementation of routine molecular profiling of patients with NSCLC using targeted NGS. We found that targeted NGS is a cost-effective and rapid platform (with a TAT of 6 days). It is feasible within the clinical workflow and enabled the detection of at least one clinically relevant genomic alterations in 62% of the analyses.

Asian people have unique clinical characteristics and tumor histology and show different prevalence of oncogenic mutations.⁽²²⁾ In this study, *EGFR* mutations were more common in women and in patients with ADC, especially with acinar predominant tumors, but less frequent in patients with solid predominant ADC. In addition, it is not correlated to age, smoking history and tumor stage. The *KRAS* mutation rate was also more common in men, ever-smokers and patients with IMA. Upon comparison of driver gene mutations of lung adenocarcinoma with the TCGA dataset, we found that *EGFR* was mutated at a much higher frequency in our cohort than in Caucasians. In contrast, *KRAS*, the second most commonly mutated gene in Caucasians, was only found in 9.8% of the Chinese ADC patients in our study. Furthermore, the subtype distribution of the *EGFR* and *KRAS* mutation was different from ethnicity. *EGFR* mutation in exon 21, *KRAS* G12D and Q61H was more frequently observed in Asians compared to Caucasians. It might be helpful to determine whether mutation phenotypes are correlated with sensitivity or resistance to *EGFR*-TKI therapy.

Another purpose of the present study was to demonstrate that our comprehensive genomic profiling assay based on a hybrid-capture NGS approach could be used to guide therapy decisions and patient enrolment into clinical trials. Screening for somatic mutations in *EGFR* and *KRAS* and rearrangements in *ALK* is now an established component of routine diagnostic practice in Chinese hospitals.⁽⁴¹⁾ However, single-gene PCR and FISH assays with limited sensitivity are more often used than NGS platforms, which are capable of identifying various alterations in multiple genes from a single tumor sample. In our study, we found that 22 (7.2%) patients harbored clinically actionable alterations that were not previously discovered in the routine clinical test, which could enable clinicians to select more targeted treatments. The majority of these alterations were recurrent gene mutations or rearrangements involving *PIK3CA*, *ROS1* and *MET*. The presence of mutations in *PIK3CA* and *MET* amplifications has been reported to possibly lead to *EGFR* TKI resistance. In our cohort, actionable genomic alterations that were potentially treatable with therapeutic agents were identified in 57% of all lung tumors and in 62% of lung ADC within nine genes (*KRAS*, *EGFR*, *ALK*, *ROS1*, *ERBB2*, *BRAF*, *PIK3CA*, *MET* and *NRAS*). A similar study previously conducted by The Lung Cancer Mutation Consortium (LCMC) showed that actionable drivers were detected in 64% (466 in 733) of lung ADC in 10 genes (*KRAS*, *EGFR*, *ALK*, *ERBB2*, *BRAF*, *PIK3CA*, *MET*, *NRAS*, *MEK1* and *AKT1*).⁽¹⁹⁾ In comparison with the LCMC study, our study had a cohort with a higher actionable mutation rate in *EGFR* and lower *KRAS* and *ALK* mutation rate. No significant difference was observed in *BRAF*, *ERBB2*, *PIK3CA*, *NRAS* and *MET* mutation status.

Table 5. Genomic alterations associated with targeted therapies

Gene	Alteration	Targeted therapy	Sensitivity or resistance	Level	Frequency
Any gene(s)					191
<i>EGFR</i>	G719X	Erlotinib, Gefitinib, Afatinib	S	I	2
	L858R	Erlotinib, Gefitinib, Afatinib	S	I	79
	L861Q	Erlotinib, Gefitinib, Afatinib	S	I	2
	Exon 19 deletion	Erlotinib, Gefitinib, Afatinib	S	I	54
	T790M	Osimertinib	S	I	1
	Exon 20 insertion	Erlotinib	R	I	2
	Amplification	Cetuximab	S ⁽²⁷⁾	III	3
<i>ERBB2</i>	Insertion	Afatinib, Dacomitinib, Trastuzumab	S ^(28–30)	II	1
<i>KRAS</i>	G12X	Erlotinib, Gefitinib	R	I	25
	G13C	Erlotinib, Gefitinib	R	I	1
	Q61X	Erlotinib, Gefitinib,	R	I	5
<i>ALK</i>	EML4-ALK	Crizotinib, Ceritinib, Alectinib	S	I	10
<i>ROS1</i>	SDC4-ROS1	Crizotinib	S	I	2
	LRIG3-ROS1	Crizotinib	S	I	1
	CD74-ROS1	Crizotinib	S	I	1
<i>MET</i>	Amplification	Erlotinib, Gefitinib	R ^(31,32)	II	3
<i>PIK3CA</i>	H1047X	Erlotinib, Gefitinib	R ^(33,34)	IV	3
<i>BRAF</i>	V600E	Vemurafenib Dabrafenib	S ^(35,36)	III	2
<i>NRAS</i>	Q61K	Trametinib	S ^(37,38)	II	1

I: Genomic alterations that are included in National Comprehensive Cancer Network (NCCN) guidelines indicating sensitivity or resistance to lung cancer therapies. II: Genomic alterations that indicate sensitivity or resistance to lung cancer therapies based on the results of phase II/III trials. III: Genomic alterations that indicate sensitivity or resistance to therapies approved by the FDA or to those included in the professional guidelines for other cancers. IV: Phase I trials or small cohort studies have indicated its effectiveness in lung cancer patients with this alteration. R, resistance; S, sensitivity.

The present study has a few limitations. First, it is a single-center analysis of the genomic profiling of lung cancer, which may not be representative of the overall situation in China. Second, although there was a higher *EGFR* mutation rate in the Chinese population, the majority of patients, including early or advanced stage patients, were still being treated with platinum therapy, mainly because TKI agents are not covered by insurance. Therefore, the clinical outcome information was available only for a relatively small subset of cases. In the future, prospective randomized clinical trials are needed to confirm the observations described in the present study.

In the present study, we revealed the similarities and differences in the mutational features of NSCLC between Chinese and Caucasian populations. We demonstrated the successful application of the hybrid capture-based NGS approach for performing comprehensive genomic profiling in Chinese lung cancer patients. Given the increased availability of various targeted therapies, our findings have implications for cancer translational research and management.

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Disclosure Statement

The authors have no conflict of interest to declare.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Targeted gene list.

Table S2. Verification of somatic mutations by IHC or FISH.

Table S3. Patients with clinical genomic alterations in more than one gene.

Fig. S1. Capture performance of 306 clinical formalin-fixed paraffin-embedded (FFPE) samples.